



D458

CRL-3632™

Description

D458 is a pediatric medulloblastoma cell line exhibiting mixed adherent and suspension morphology that was isolated from a 7 year old female patient.

Organism: *Homo sapiens*, human

Tissue: Brain

Age: 6 years

Gender: Male

Morphology: Epithelial-like and/or Rounded

Growth properties: Adherent

Disease: Medulloblastoma

Cells per vial: Approximately 2.0 to 3.0 x 10⁶

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to

understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Atmosphere: 95% Air, 5% CO₂

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the

cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium:

The base medium for this cell line is Dulbecco's Modified Eagle's Medium (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium:

- Fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 10%
- L-Glutamine (ATCC 30-2214) to a final concentration of 2 mM

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70° C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 to 400 x *g* for 5 to 12 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37° C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase

amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Collect the medium with the suspension cells, and recover the cells by centrifugation.
2. Gently rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
3. Note: Practice care when rinsing with PBS as cells may detach during the wash step. If cells dislodge, collect the PBS with the cell supernatant for subculturing. Prepare 0.125% Trypsin-0.265 mM EDTA by aseptically combining the following components: • 50 mL 0.25% Trypsin/0.53 mM EDTA (ATCC catalog # 30-2101) • 50 mL PBS (ATCC catalog # 30-2200) Add 2.0 to 3.0 mL of 0.125%Trypsin-0.265mM EDTA to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium, aspirate cells by gently pipetting, and combine with the floating cells recovered above.
5. Centrifuge the cell suspension at approximately 125 to 400 x *g* for 5 to 12 minutes to remove dissociation agent.
6. Resuspend the cell pellet in an appropriate amount of complete culture medium
7. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 6.0×10^4 and $1.06.0 \times 10^5$ viable cells/cm².
8. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 5.0×10^4 and 1.0×10^5 cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:88 is recommended

Medium Renewal: Every 2 to 3 days

Reagents for cryopreservation: Complete Culture Medium + 5% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: D458 (ATCC CRL-3632)

References

References and other information relating to this material are available at www.atcc.org.

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